Scientists ratchet up understanding of cellular protein factory

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LANL theorists use Encanto supercomputer to unravel ribosome mystery

LOS ALAMOS, New Mexico, December 2, 2010—Theoretical biologists at Los Alamos National Laboratory have used a New Mexico supercomputer to aid an international research team in untangling another mystery related to ribosomes—those enigmatic jumbles of molecules that are the protein factories of living cells. The research, published today in the journal *Nature*, could aid in development of new antibiotics used to fight multidrug resistant superbugs such as MRSA (methicillin-resistant *Staphylococcus aureus* infections) found in many U.S. hospitals. The work may also be important for combating engineered strains of anthrax and plague.

In the context of synthetic biology, understanding the ribosome could be key to developing nanofactories that produce designer biomolecules and polymers.

In the paper, "*Head swivel on the ribosome facilitates translocation via intra-subunit tRNA hybrid sites*," Los Alamos National Laboratory researchers Karissa Sanbonmatsu and Paul Whitford and José N. Onuchic at the University of California-San Diego join Christian Spahn, Andreas Ratje, and others from the Institute for Medical Physics and Biophysics, Berlin, Germany, to describe for the first time how a complicated swivel movement within a bacterial ribosome accommodates synthesis of proteins.

Ribosomes are composed of long chemical chains, called ribonucleic acids (RNA), and proteins. Each ribosome has two interlocked subunits, one large and one small, which behave as a single molecular machine. Because of its makeup, each ribosome resembles a tangle of threads or a handful of rubber bands tossed together. Despite the ribosome's outwardly disjointed appearance, researchers have found that the two subunits ratchet, un-ratchet, and swivel during protein synthesis to allow introduction of helper chemicals called transfer RNAs (tRNAs) into its folds to manufacture new chains of protein molecules. The proteins are used to create new cells or perform necessary functions within the host cell or organism.

Ribosomes build proteins by linking chemical segments fashioned from instructions delivered via messenger RNA, which is DNA's molecular cousin. Each segment, or amino acid, corresponds to a trio of bases in the message that, in turn, complement trios encoded in transfer RNA. Each base in the trio corresponds to a single chemical complement found on the RNA. In order for protein synthesis to occur, the tRNA must

bind to the ribosome at two distinct sites—one to decode the information and another to link the new amino acid to the emerging protein.

After each amino acid is added, the ribosome must crawl along the message to create additions. Exactly how this crawling occurs has been a mystery for several decades. Researchers have suspected that ratcheting motions of the two ribosomal subunits are key to allowing RNA and associated catalysts into the complex structure of the ribosome so the RNA and ribosome can couple at the crucial sites to create proteins. In the *Nature* paper, the researchers discovered that the majority of crawling (movement along messenger RNA) occurs during a new kind motion, “head swivel,” rather than ratcheting.

The paper describes how an antibiotic was used to inhibit the full swivel and ratcheting motion of a ribosome from a bacterium called *Thermus Thermophilus*, which thrives in hot acidic environments. The ribosomes were flash-frozen at various mid-swivel and mid-ratchet configurations and examined under a powerful electron microscope.

The observed configurations were then coupled with a computer model newly developed at Los Alamos called MDFIT. The computer algorithm integrates molecular simulation with maps of ribosome structures obtained through the cryogenic microscopy. The Los Alamos team then used the Encanto supercomputer—funded by the state of New Mexico and housed at the Intel plant in Rio Rancho—to create molecular snapshots of the complicated motion of the ribosomal subunits during protein synthesis.

Previously, scientists were only able to observe the beginning or end states of the motion. These new images show the behavior of the ribosome through its range of motion—much like early photographic motion studies that showed the entire fluid movement of a galloping horse. In addition to showing the importance of head swivel motion, the study showed that a key catalyst in the process acts as a dynamic pawl in the ribosomal machinery, providing directionality and acceleration for translocation of the tRNA. The understanding provided by the new model will help researchers to develop more effective antibiotics that target the ribosomal machinery of harmful organisms.

“While static images of the ribosome have revealed the detailed structure of the complex, we still don’t know how all the parts of the machine work together to make proteins,” said Janna Wehrle, Ph.D., who oversees Dr. Sanbonmatsu’s and other structural biology grants at the National Institutes of Health. “By showing how the bacterial ribosome carries out a key step of protein synthesis, this study has begun to produce a more dynamic picture while offering a new way to target harmful, multi-drug resistant bacteria.”

The research team includes: Andreas H. Ratje, Justus Loerke, Matthias Brünner, Peter W. Hildebrand, Thorsten Mielke and Christian M.T. Spahn, Institute of Medical Physics and Biophysics, Berlin; Aleksandra Mikolajka, Agata L. Starosta, Alexandra Dönhöfer and Daniel N. Wilson, Ludwig-Maximilians University, Munich; Sean R. Connell and Paola Fucini, Goeth University, Frankfurt; Paul C. Whitford and Karissa Y. Sanbonmatsu, Los Alamos National Laboratory, Los Alamos, New Mexico; José N. Onuchic, University of California-San Diego; Yanan Yu, Florida State University, Tallahassee, Florida; Roland K. Hartmann, Institute for Pharmaceutical Chemistry, Marburg, Germany; Pawel A. Penczek, University of Texas, Houston Medical School.


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